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PLAQUE FORMATION BY PSITTACOSIS VIRUS

ABSTRACT

The production of plaques by psittacosis virus on monolayers of McCoy cells is described in this preliminary report. Plaques appeared in five to seven days and ranged in diameter from 0.2 to 0.5 millimeter. Formation of plaques was inhibited by psittacosis antiserum, which confirmed their virus specificity. The relationship between plaque numbers and virus concentration was linear.

Although the production of plaques on cell monolayers has been reported for a considerable number of viral agents,[1] it has been demonstrated for only one member of the psittacosis group, meningopneumonitis virus.[2,3] This preliminary communication describes plaque formation by another member, psittacosis (Borg) virus.

Cultures of an established cell line, McCoy, originally derived from human synovial tissue[4] and shown to be 99 per cent susceptible to infection with psittacosis virus,[5] were suspended in nutrient medium composed of mixture 199 with 0.5 per cent lactalbumin hydrolyzate (LAH), 10 per cent heat-inactivated calf serum, 50 micrograms of streptomycin, and 75 micrograms of kanamycin. Five milliliters of suspension containing 1.5×10^6 cells was dispensed into two-ounce Brockway bottles (Sani-Glas, Brockway Glass Co.), capped, and incubated at 35°C. Continuous cell monolayers were formed, usually within 48 hours. These were washed once with ten milliliters of phosphate-buffered saline (PBS) and once with five milliliters of maintenance medium (mixture 199 with 5 per cent calf serum). A virus suspension that had been passed five times previously on McCoy cell cultures was employed in 0.5-milliliter volumes to infect cell monolayers. After an adsorption time of four hours at 35°C, residual inoculum was removed. Four milliliters of primary overlay medium, heated to 45°C, was added and cell monolayers were incubated four days at 35°C. The overlay medium contained one part calf serum, ten parts 2.2 per cent Noble agar (Difco), and ten parts nutrient medium. The latter consisted of one per cent LAH, 0.2 per cent yeast extract, and 0.04 per cent streptomycin in twofold concentrated Earle's salt solution. One milliliter of second overlay medium, differing only from primary overlay medium by the addition of 0.015 per cent neutral red, was added onto cell monolayers that were incubated an additional one to three days at 35°C.

On the fifth day, but more commonly on the sixth day, after cell monolayers were exposed to virus suspension, minute macroscopic plaques appeared as clear discrete areas ranging in diameter from 0.2 to 0.5 millimeter (Figure 1). Plaques examined microscopically consisted of unstained cells in a degenerate condition surrounded by a well-defined periphery of healthy stained cells. Although the size of plaques remained constant on subsequent incubation, their number increased gradually and reached a maximum on the seventh day. Enumeration was facilitated by employing a dissecting microscope at magnification 15X. By the tenth day, plaques tended to fade because of cell degeneration. Plaques produced by psittacosis virus were similar in size and appearance to those described for meningopneumonitis virus on strain L cell monolayers.[3] The maximum diameters of plaques produced by meningopneumonitis virus was attained on the eleventh day,[3] which suggests a difference between the rates of plaque formation by the two viruses. This may be related, however, to the use of different cell lines.

Plaque formation by different concentrations of virus was linear over a dilution range of 1.5 log units (Figure 2), suggesting that each plaque was initiated by a single virus particle. Significant reductions in plaque formation by varied dilutions of rooster psittacosis antiserum mixed with a standard suspension of virus confirmed the virus specificity of plaques.

In sensitivity, the plaque assay compared favorably with another technique, that of yolk-sac inoculation of seven-day chick embryonated eggs, based on dilution end point. Mean values of replicate determinations made by both techniques with a standard virus suspension were 2.7×10^6 plaque-forming units (PFU) and 2.8×10^6 egg LD_{50} per milliliter, respectively. The feasibility of employing a plaque assay for psittacosis virus is indicated by these preliminary findings.

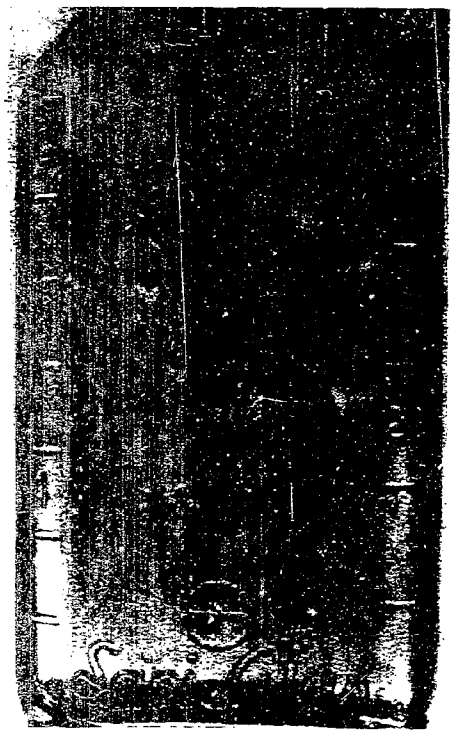

Figure 1. Plaques Formed by Psittacosis Virus on McCoy Cell Monolayers Six Days after Infection; Magnification 1X.

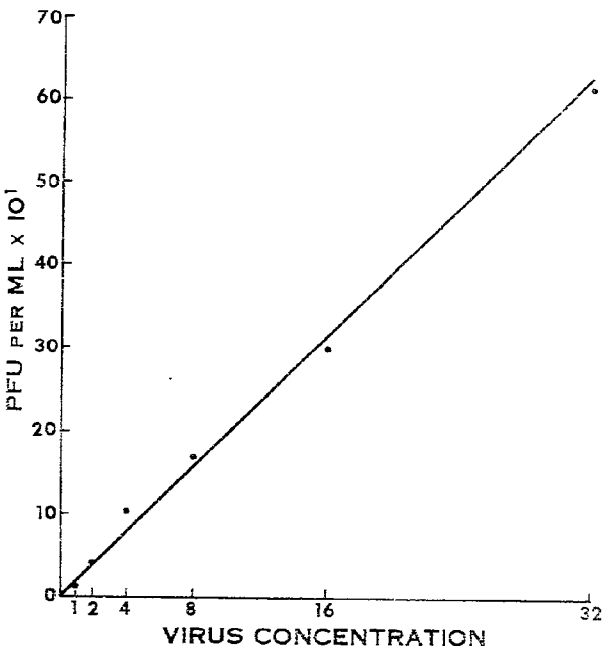

Figure 2. Linear Function Between Number of Plaques Formed on McCoy Cell Monolayers and Concentration of Psittacosis Virus. Each point is the mean of three determinations.

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